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# A NOVEL METHOD OF DIAGNOSING,
## MONITORING, AND STAGING PROSTATE CANCER

## FIELD OF THE INVENTION

This invention relates, in part, to newly developed
5  assays for detecting, diagnosing, monitoring, staging, and
prognosticating cancers, particularly prostate cancer.

## BACKGROUND OF THE INVENTION

Cancer of the prostate is the most prevalent
malignancy in adult males, excluding skin cancer, and is an
10  increasingly prevalent health problem in the United States.
In 1996, it was estimated that in the United States, 41,400
deaths would result from this disease, indicating that
prostate cancer is second only to lung cancer as the most
common cause of death in the same population.  If diagnosed
15  and treated early, when the cancer is still confined to the
prostate, the chance of cure is significantly higher.

Treatment decisions for an individual are linked to
the stage of prostate cancer present in that individual.  A
common classification of the spread of prostate cancer was
20  developed by the American Urological Association (AUA).  The
AUA classification divides prostate tumors into four stages,
A to D.  Stage A, microscopic cancer within prostate, is
further subdivided into stages A1 and A2.  Sub-stage A1 is a
well-differentiated cancer confined to one site within the
25  prostate.  Treatment is generally observation, radical
prostatectomy, or radiation.  Sub-stage A2 is a moderately to
poorly differentiated cancer at multiple sites within the
prostate.  Treatment is radical prostatectomy or radiation.
Stage B, palpable lump within the prostate, is further
30  subdivided into stages B1 and B2.  In sub-stage B1, the cancer
forms a small nodule in one lobe of the prostate.  In sub-
stage B2, the cancer forms large or multiple nodules, or
occurs in both lobes of the prostate. Treatment for both sub-
stages B1 and B2 is either radical prostatectomy or radiation.

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Stage C is a large cancer mass involving most or all of the prostate and is further subdivided into two stages. In sub-stage C1, the cancer forms a continuous mass that may have extended beyond the prostate. In sub-stage C2, the cancer

5   forms a continuous mass that invades the surrounding tissue. Treatment for both these sub-stages is radiation with or without drugs. The fourth stage is metastatic cancer and is also subdivided into two stages. In sub-stage D1, the cancer appears in the lymph nodes of the pelvis. In sub-stage D2,

10  the cancer involves tissues beyond lymph nodes. Treatment for both these sub-stages is systemic drugs to address the cancer as well as pain.

        However, current prostate cancer staging methods are limited. As many as 50% of prostate cancers initially staged

15  as A2, B, or C are actually stage D, metastatic. Discovery of metastasis is significant because patients with metastatic cancers have a poorer prognosis and require significantly different therapy than those with localized cancers. The five year survival rates for patients with localized and metastatic

20  prostate cancers are 93% and 29%, respectively.

        Accordingly, there is a great need for increasingly sensitive methods for the staging of a cancer in a human to determine whether or not such cancer has metastasized and for monitoring the progress of a cancer in a human.

25          In the present invention, methods are provided for detecting, diagnosing, monitoring, staging and prognosticating cancers, particularly prostate cancer via seven (7) Prostate Specific Genes (PSG). The seven PSGs refer, among other things, to native proteins expressed by the genes comprising

30  the polynucleotide sequences of any of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7. In the alternative, what is meant by the seven PSGs as used herein, means the native mRNAs encoded by the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7 or levels of the genes comprising

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any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7.

5    Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description.   It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only.  Various changes and modifications within the spirit and scope of the

10   disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

## SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the

15   present invention to provide a method for diagnosing the presence of prostate cancer in a patient which comprises measuring levels of PSG in a sample of cells, tissue or bodily fluid from the patient and comparing the measured levels of PSG with levels of PSG in preferably the same cells, tissue,

20   or bodily fluid type of a control, wherein an increase in the measured PSG levels in the patient versus levels of PSG in the control is associated with prostate cancer.

Another object of the present invention is to provide a method of diagnosing metastatic prostate cancer in

25   a patient which comprises measuring PSG levels in a sample of cells, tissue, or bodily fluid from the patient and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured PSG levels in the patient versus

30   levels of PSG in the control is associated with a cancer which has metastasized.

Another object of the present invention is to provide a method of staging prostate cancer in a patient which comprises identifying a patient having prostate cancer,

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measuring levels of PSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue or bodily fluid type of a control. An increase

5   in measured PSG levels in the patient versus PSG levels in the control can be associated with a cancer which is progressing while a decrease or equivalent level of PSG measured in the patient versus the control can be associated with a cancer which is regressing or in remission.

10          Another object of the present invention is to provide a method of monitoring prostate cancer in a patient for the onset of metastasis. The method comprises identifying a patient having prostate cancer that is not known to have metastasized, periodically measuring levels of PSG in a sample

15  of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured PSG levels versus control PSG levels is associated with a cancer which has

20  metastasized.

          Yet another object of the present invention is to provide a method of monitoring the change in stage of prostate cancer in a patient which comprises identifying a patient having prostate cancer, periodically measuring levels of PSG

25  in a sample of cells, tissue, or bodily fluid obtained from the patient, and comparing the measured PSG levels with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a control wherein an increase in measured PSG levels versus the control PSG levels is associated with a cancer

30  which is progressing and a decrease in the measured PSG levels versus the control PSG levels is associated with a cancer which is regressing or in remission.

          Other objects, features, advantages and aspects of the present invention will become apparent to those of skill

35  in the art from the following description. It should be

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understood, however, that the following description and the
specific examples, while indicating preferred embodiments of
the invention, are given by way of illustration only. Various
changes and modifications within the spirit and scope of the
5    disclosed invention will become readily apparent to those
skilled in the art from reading the following description and
from reading the other parts of the present disclosure.


**DESCRIPTION OF THE INVENTION**

The present invention relates to diagnostic assays
10   and methods, both quantitative and qualitative for detecting,
diagnosing, monitoring, staging, and prognosticating cancers
by comparing levels of PSG measured in a patient with levels
of PSG in a control. What is meant by "levels of PSG" as used
herein, means levels of the native protein expressed by the
15   gene comprising the polynucleotide sequence of any of SEQ ID
NO: 1, 2, 3, 4, 5, 6 or 7. In the alternative, what is meant
by "levels of PSG" as used herein, is levels of the native
mRNA encoded by the gene comprising any of the polynucleotide
sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6 or 7 or levels of the
20   gene comprising any of the polynucleotide sequence of SEQ ID
NO: 1, 2, 3, 4, 5, 6 or 7. Such levels are preferably
measured in at least one of cells, tissues and/or bodily
fluids, and includes determination of both normal and abnormal
levels of PSGs. Thus, for instance, a diagnostic assay in
25   accordance with the invention for diagnosing overexpression
of PSG protein compared to control bodily fluids, cells, or
tissue samples may be used to diagnose the presence of
cancers, including prostate cancer. Any of the seven PSGs may
be measured alone in the methods of the invention, all
30   together or in various combinations of the seven PSGs.

By "control" it is meant a human patient without
cancer and/or non cancerous samples from the patient, also
referred to herein as a normal human control; in the methods
for diagnosing or monitoring for metastasis, control may also

include samples from a human patient that is determined by reliable methods to have prostate cancer which has not metastasized.

5       All the methods of the present invention may optionally include measuring the levels of other cancer markers as well as PSG. Other cancer markers, in addition to PSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art. For example, simultaneous testing for increases in PSA as well as

10      increases in PSG are also within the scope of the present invention and believed to provide a higher level of assurance that such cancer being tested is metastatic or the onset of metastasis has occurred.

## Diagnostic Assays

15      The present invention provides methods for diagnosing the presence of prostate cancer by analyzing for changes in levels of PSG in cells, tissues or bodily fluids compared with levels of PSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase

20      in levels of PSG in the patient versus the normal human control is associated with the presence of prostate cancer. Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells,

25      tissues, or bodily fluid levels of the cancer marker, such as PSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal human control.

        The present invention also provides a method of

30      diagnosing metastatic prostate cancer in a patient having prostate cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a human cancer patient suspected of having prostate cancer which may have metastasized (but which was not previously known to

35      have metastasized) is identified. This is accomplished by a

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variety of means known to those of skill in the art. For example, in the case of prostate cancer, patients are typically diagnosed with prostate cancer following traditional detection methods.

5          In the present invention, determining the presence of PSG in cells, tissues, or bodily fluid, is particularly useful for discriminating between prostate cancer which has not metastasized and prostate cancer which has metastasized.

          Existing techniques have difficulty discriminating
10   between prostate cancer which has metastasized and prostate cancer which has not metastasized and proper treatment selection is often dependent upon such knowledge.

          In the present invention, the cancer marker levels measured in such cells, tissue, or bodily fluid are PSGs, and
15   are compared with levels of PSG in preferably the same cells, tissue, or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just PSG in serum, this level is preferably compared with the level of PSG in serum of a normal human patient. An increase in the PSG in
20   the patient versus the normal human control is associated with prostate cancer which has metastasized.

          Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored
25   has metastasized is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as PSG, are at least two times higher, and most preferable are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal patient.

30   **Staging**

          The invention also provides a method of staging prostate cancer in a human patient.

          The method comprises identifying a human patient having such cancer and analyzing a sample of cells, tissues,
35   or bodily fluid from such patient for PSG. Then, the method

compares PSG levels in such cells, tissues, or bodily fluid with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in PSG levels in the patient versus the normal

5 human control is associated with a cancer which is progressing and a decrease in the levels of PSG is associated with a cancer which is regressing or in remission.

*Monitoring*

Further provided is a method of monitoring prostate

10 cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for PSG; and comparing the PSG levels

15 in such cells, tissue, or bodily fluid with levels of PSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in PSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

20 Further provided by this invention is a method of monitoring the change in stage of prostate cancer in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissue, or bodily fluid from such patient for PSG;

25 comparing the PSG levels in such cells, tissue, or bodily fluid with levels of PSG in preferably the same patient.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the

30 particular patient, and the stage of the cancer.

*Assay Techniques*

Assay techniques that can be used to determine levels of gene expression, such as PSG of the present invention, in a sample derived from a host are well-known to those of skill

35 in the art. Such assay methods include radioimmunoassays,

reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses and ELISA assays. Among these, ELISAs are frequently preferred to

5  diagnose a gene's expressed protein in biological fluids. An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to PSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to

10  PSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.

To carry out the ELISA, antibody specific to PSG is

15  incubated on a solid support, e.g., a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time PSG binds

20  to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to PSG and linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to PSG.

25  Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to PSG antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to

30  the amount of PSG protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to PSG attached to a solid support and

35  labeled PSG and a sample derived from the host are passed over

the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of PSG in the sample.

Nucleic acid methods may be used to detect PSG mRNA
5   as a marker for prostate cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-
10  transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse
15  transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of
20  cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e., gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the PSG gene is
25  fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the PSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy
30  of the RNA, isolated from the tissue of interest.

Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule
35  designed to detect the hybrid. Quantitation of the level of

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gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and
5  then using that material to generate a standard curve.

The above tests can be carried out on samples derived from a variety of patients' cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) such as from tissue biopsy and autopsy material. Bodily fluids useful in
10 the present invention include blood, urine, saliva, or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum, or any derivative of blood.

**EXAMPLES**

15      The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or
20 circumscribe the scope of the disclosed invention.

**EXAMPLE 1: PSGs**

Searches were carried out and PSGs identified using the following Search Tools as part of the LIFESEQ® database available from Incyte Pharmaceuticals, Palo Alto, CA:
25      1.    Library Comparison (compares one library to one other library) allows the identification of clones expressed in tumor and absent or expressed at a lower level in normal tissue.

2.    Subsetting is similar to library comparison but
30 allows the identification of clones expressed in a pool of libraries and absent or expressed at a lower level in a second pool of libraries.

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3.     Transcript Imaging lists all of the clones in a single library or a pool of libraries based on abundance. Individual clones can then be examined using Electronic Northerns to determine the tissue sources of their component
5  ESTs.

4.     Protein Function: Incyte has identified subsets of ESTs with a potential protein function based on homologies to known proteins.  Some examples in this database include Transcription Factors and Proteases.  Some leads were
10  identified by searching in this database for clones whose component ESTs showed disease specificity.

Electronic subtractions, transcript imaging and protein function searches were used to identify clones, whose component ESTs were exclusively or more frequently found in
15  libraries from specific tumors.  Individual candidate clones were examined in detail by checking where each EST originated.

**Table 1:**

| SEQ ID NO: | Clone ID # | Gene ID # | |
|---|---|---|---|
| 1 | 1550426 | 244673 | Protein Function (Transcription Factors) |
| 2 | 1255804 | 14878 | Subsetting |
| 3 | 1808432 | 255819 | Subsetting |
| 4 | 3930803 | none | Subsetting |
| 5 | 645804 | 235032 | Subsetting |
| 6 | 1862352 | 221558 | Subsetting |
| 7 | 1450626 | 236019 | Subsetting |

**EXAMPLE   2:     Measurement of SEQ ID NO:1; Clone ID # 1550426; Gene ID #244673 (pro101)**

30       The example is carried out using standard techniques, which are well known and routine to those of skill in the art,

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except where otherwise described in detail. Routine molecular biology techniques of the following example are carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold 5 Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).


### *Relative Quantitation of Gene Expression*

Real-time quantitative PCR with fluorescent Taqman probes is a quantitative detection system utilizing the 5'- 10 3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected 15 by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either 20 cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample are used as the basis for comparative results (calibrator). 25 Quantitation relative to the "calibrator" is obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

To evaluate the tissue distribution, and the level of pro101 (SEQ ID NO:1) in normal and tumor tissue, total RNA 30 was extracted from tumor and matched normal adjacent tissues and from unmatched tumor and normal tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction carried out using primers and Taqman probe specific to pro101 (SEQ ID NO:1). The results

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were obtained using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of pro101 (SEQ ID NO:1) compared to the calibrator.

5    The absolute numbers are depicted in the following Table 2 as relative levels of expression in 12 normal tissues of pro101 (SEQ ID NO:1) compared to kidney (calibrator). These RNA samples were generated by pooling samples from a particular tissue from different individuals.

**Table 2: Relative levels of pro101 Expression in Pooled**
10        **Samples**

| Tissue | NORMAL |
|--------|--------|
| Brain | 1.2 |
| Heart | 2 |
| Kidney | 1 |
| Liver | 7.2 |
| Lung | 48.2 |
| Mammary | 2.5 |
| Prostate | 1418.4 |
| Spleen | 1.6 |
| Small | 1.9 |
| Testis | 57.3 |
| Thymus | 1.3 |
| Uterus | 7.6 |

The relative levels of expression in Table 2 show that for the
25   PSG pro101 (SEQ ID NO:1) mRNA expression is more than 20 fold higher in the pool of normal prostate compared with the other 11 normal tissue pools analyzed. These results demonstrate that mRNA expression of the PSG is highly specific for prostate.

30        The tissues shown in Table 2 correspond to pools of samples from different individuals. The tissues shown in the following Table 3 were obtained from individuals and are not pooled. Hence the values for mRNA expression levels shown in Table 2 cannot be directly compared to the values shown in
35   Table 3.

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The absolute numbers in Table 3 are relative levels of expression of pro101 (SEQ ID NO:1) compared to kidney (calibrator), in 60 pairs of matching samples. Each matching pair contains the cancer sample for a particular tissue and the normal adjacent sample for that same tissue from the same individual. The results from 3 unmatched ovary tumor, 3 unmatched normal ovary, 1 unmatched mammary tumor and 1 unmatched normal mammary gland are also shown.

**Table 3: Relative Levels of pro101 Expression in Individual Samples**

| TISSUE | CANCER | MATCHING | UNMATCHED |
|--------|--------|----------|-----------|
| Prostate 1 | 103.9 | 0 | |
| Prostate 2 | 2219 | 84.2 | |
| Prostate 3 | 5048.2 | 3623.6 | |
| Prostate 4 | 11052.3 | 2029.4 | |
| Prostate 5 | 229.1 | 41.1 | |
| Prostate 6 | 57.9 | 25.3 | |
| Prostate 7 | 58.5 | 57.069 | |
| Prostate 8 | 1074.6 | 610.8 | |
| Prostate 9 | 32.7 | 79.3 | |
| Prostate 10 | 15.8 | 2.09 | |
| Prostate 11 | 436.4 | 438 | |
| Prostate 12 | 49.5 | 59.3 | |
| Prostate 13 | 128 | 56 | |
| Bladder 1 | 0 | 0 | |
| Bladder 2 | 0 | 0 | |
| Bladder 3 | 0.7 | 0 | |
| Colon 1 | 0 | 0 | |
| Colon 2 | 0 | 0 | |
| Colon 3 | 0 | 0 | |
| Colon 4 | 3.3 | 1.9 | |
| Colon 5 | 0.1 | 0.8 | |
| Colon 6 | 0 | 0 | |
| Lung 1 | 0 | 0 | |
| Lung 2 | 0.5 | 1.6 | |
| Lung 3 | 1.4 | 2.1 | |
| Lung 4 | 0 | 0 | |
| Lung 5 | 0 | 0 | |
| Kidney 1 | 0 | 0 | |
| Kidney 2 | 0 | 0 | |
| Kidney 3 | 0 | 0 | |
| Kidney 4 | 0 | 0 | |
| Liver 1 | 1.5 | 5.7 | |
| Liver 2 | 26.9 | 7.9 | |
| Liver 3 | 0 | 0 | |

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| | | | |
|---|---|---|---|
| Pancreas 1 | 0.9 | 0.9 | |
| Pancreas 2 | 3 | 0 | |
| Pancreas 3 | 0 | 0 | |
| Pancreas 4 | 0 | 0 | |
| Pancreas 5 | 0 | 0 | |
| Stomach 1 | 0 | 0 | |
| Stomach 2 | 0 | 0 | |
| Stomach 3 | 0 | 0 | |
| Stomach 4 | 0 | 0 | |
| Stomach 5 | 0 | 0 | |
| Sm Int 1 | 0 | 0 | |
| Sm Int 2 | 0 | 0 | |
| Testis 1 | 0 | 0 | |
| Mammary 1 | 4 | 0 | |
| Mammary 2 | 5.6 | 0 | |
| Mammary 3 | 0.5 | 0 | |
| Mammary 4 | 0.4 | 0 | |
| Mammary 5 | 0.5 | | |
| Mammary 6 | | | 0 |
| Endo 1 | 1.6 | 7.6 | |
| Endo 2 | 0 | 0 | |
| Endo 3 | 0 | 0 | |
| Endo 4 | 0.3 | 0.2 | |
| Endo 5 | 5.8 | 5 | |
| Uterus 1 | 0 | 0 | |
| Uterus 2 | 0 | 0 | |
| Uterus 3 | 0 | 0 | |
| Uterus 4 | 2.2 | 2.6 | |
| Ovary 1 | 1.4 | | |
| Ovary 2 | | | 11.6 |
| Ovary 3 | 1.5 | | |
| Ovary 4 | | | 22.9 |
| Ovary 5 | 0 | | |
| Ovary 6 | | | 1.8 |

Among 128 samples in Table 3 representing 14 different
tissues, the higher levels of expression are consistently in
prostate tissues. These results confirm the tissue
specificity results obtained with normal samples shown in
Table 2. Table 2 and Table 3 represent a combined total of
140 samples in 18 human tissue types. Sixty-eight samples
representing 13 different tissue types excluding prostate had
no detected pro101 mRNA (Table 3). In 4 tissues (stomach
small intestine kidney and testis) no pro101 (SEQ ID NO:1)
mRNA was detected for any sample tested from individuals
(Table 3). Expression of this PSG was detected in testis in
the pooled normal sample (Table 3). The median expression in

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prostate cancer samples in Table 3 is 166.5 units.  Excluding
Ovary 4 (Normal), only 1 sample in Table 3, Liver 2 (Cancer),
is greater than 10% of this value.

5      Comparisons of the level of mRNA expression in
prostate tumor samples and the normal adjacent tissue from the
same individuals are also shown in Table 3.  The PSG pro101
(SEQ ID NO:1) is expressed at higher levels in 9 of 13 (69%)
prostate cancer tissues (Prostate 1, 2, 3, 4, 5, 6, 8, 10 and
13) compared with the corresponding normal adjacent tissue.
10 The level of expression of this PSG is lower in prostate tumor
compared to normal adjacent tissue in two samples (Prostate
9 and 12).  Equivalent levels of expression were detected in
two matched samples (Prostate 7 and 11).  Previous mRNA
expression analysis for genes coding for the diagnostic
15 markers PSA and PLA2 showed higher expression of the mRNA in
40% to 80% of the tumor samples compared to matching normal
adjacent tissue.  Higher expression in the tumor sample
compared to the corresponding normal adjacent tissue is
observed for Bladder 3, Colon 4, Liver 2, Pancreas 2,
20 Endometrium 5 and. Mammary 1, 2 and 3.  Higher expression in
the normal adjacent samples is observed for Colon 5, Lung 2,
Lung 3, Liver 1, Endometrium 1 and Uterus 4.  However, the
levels detected are in most cases comparable amongst the
different tissues and low compared to levels found in most
25 prostate tissues.

The high level of tissue specificity, plus the mRNA
overexpression in 9 of 13 of the prostate tumor samples tested
compared to the normal adjacent tissues are believed to make
the PSG, pro101 (SEQ ID NO:1) a good diagnostic marker for
30 detection of prostate cancer using mRNA.